Applicant: Vincent P. Stanton, Jr.

Serial No.: 09/697,028 Filed: October 25, 2000

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Replace the paragraph beginning at page 28, line 8, with the following rewritten paragraph:

ket No.: 11926-112001

--Figure 4. In this figure, an alternative method of primer design in the present invention involves the use of a primer with an internal loop. The primer is designed (primer R1) such that one of the bases corresponding to the native sequence is removed and replaced with a loop. In this case the G/C indicated by the arrow below the target sequence is replaced with the recognition sequence for Fok I and Fsp I. Upon hybridization to the DNA template, the primer will form a loop structure. This loop will be incorporated into the amplicon during the amplification process, thereby introducing the Fok I and Fsp I restriction sites (indicated by the box). The resultant amplicon is incubated with Fok I and Fsp I under optimal digestion conditions producing an 8-mer and a 12-mer fragment. As in Figure 1, the 12-mer contains the polymorphic base (A, underlined) and can be analyzed by mass spectrometry to identify the base at the polymorphic site.--

Replace the paragraph beginning at page 28, line 20, with the following rewritten paragraph:

of target DNA is shown. As is depicted in figures 1-4 for the enzyme pair FspI/FokI; in this figure, PvuII/FokI restriction enzymatic sites can be incorporated in the same manner as previously described for Figures 1-4. A primer is designed such that a BsgI/PvuII sites form a hair-pin loop when the primer is hybridized to the target DNA sequence. After amplification by PCR, the resultant amplicon will have the PvuII/FokI sites incorporated in the resultant amplicon (as indicated by the boxed sequence). After digestion under conditions optimal for PvuII and BsgI, the resultant fragments, an 14 mer and a 16 mer, are sufficient for mass spectrometric analysis and the polymorphic site is contained in the 16mer (A, underlined).--

Replace the paragraph beginning at page 28, line 30, with the following rewritten paragraph:

--Figure 6. Shown in this figure is an alternative restriction enzyme pair for the preparation of fragments containing the polymorphic site for mass spectrometric analysis. PvuII/FokI restriction enzyme recognition sites form a hair-pin loop when hybridized to the target DNA sequence. After amplification by PCR, the resultant amplicon will have the PvuII/FokI sites incorporated in the resultant amplicon (as indicated by the boxed sequence). After digestion





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under conditions optimal for PvuII and FokI restriction, the resultant fragments, an 16 mer and a 20 mer, are sufficient for mass spectrometric analysis and the polymorphic site is contained in the 20mer (A, underlined) .--

ket No.: 11926-112001

Replace the paragraph beginning at page 29, line 26, with the following rewritten paragraph:

-- Figure 9. Shown in this figure is an example of the utility in the present invention of including a restriction enzyme recognition site for which the restriction enzyme creates a nick in the DNA amplicon instead of causing a double strand break. As shown in this figure, a primer R is designed to incorporate a N.BstNB I recognition site (GAGTCNNNN^NN) in addition to a FokI restriction site. As in previous figures, the primer forms a hair-pin loop structure when hybridized to the target DNA region, however, the PCR amplicon has the incorporated restriction site sequences. Digestion with FokI and N.BstNB I results in a 10 mer fragment that contains the polymorphic base (T, underlined). Such a fragment is sufficient for analysis using a mass spectrometer.--

In the claims:

Please cancel claims 1-9.

Please add new claims 10-16.

- --10. A method for biasing a DNA amplification reaction such that a first nucleic acid molecule having a first nudeotide present at a polymorphic site is amplified to a greater extent than a second nuclei acid molecule having a second, different nucleotide present at the polymorphic site, comprising
- (a) contacting a sample of DNA comprising at least the first nucleic acid molecule with two amplification primers that hybridize to both the first nucleic acid molecule and the second nucleic acid molecule at locations which flank the polymorphic site, one of the two primers including a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic

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